



## Review Article

# Green Synthesis of Ag and Au Nanoparticles from Micro and Macro Algae - Review

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**Abstract:** The production of nanostructures, nanocomposites and modified nanostructures for water remediation will increase because of the need for producing clean water in fast and low energy consumption ways. Nanoparticles are widely used various fields such as electronics, cosmetics, water purification, biomedical, and biotechnology. Nanoparticles can be synthesized by physical methods, chemical and biological methods. Biosynthesis of nanoparticles using biological agents have gained much attention in the area of nanotechnology in last few decades because of cost effective, nontoxic, and ecofriendly. Algae have been used to reduce metal ions and subsequently for the biosynthesis of nanoparticles. The present review is devoted to the possibility of metal nanoparticle synthesis using alga extract. The important advantages of these biological systems are an ecofriendly, economical, high-yielding, expeditious and energy-efficient method. This review is mainly focused on recent progress on the utilization of algae of various classes, for the synthesis of Silver and Gold nanoparticles, their characterization and possible mechanisms.

**Keywords:** Nanoparticles, Microalgae, Macroalgae, Wastewater, Biosynthesis

## 1. Introduction

In recent years, nanotechnology is an escalating field of modern research involving in synthesis design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometer scale [1, 2]. Water purification using Nano-filtration technology or through adsorption and catalytic degradation processes was made possible by the advances achieved and mysteries revealed in the quantum world. Worldwide, the need for clean water is increasing because of population increase, drought and the contamination of conventional water sources. The innovation of new technologies to increase the availability of clean water commenced 40 years ago with the establishment of three membrane separation processes; reverse osmosis (RO), ultrafiltration (UF) and microfiltration (MF). During the 1970s and 1980s, nanofiltration membranes (Loose RO) were developed as an intermediate filtration material between

ultrafiltration and reverse osmosis. Membrane processes using different types of membrane are becoming increasingly popular for the production of drinking water from seawater, brackish water, wastewater, surface water and groundwater [3-10].

Nanotechnology is being applied in the production of water purification membranes. The production of nanostructures, nanocomposites and modified nanostructures for water remediation will increase because of the need for producing clean water in fast and low energy consumption ways. NPs are categorized into three types: natural nanoparticles, incidental nanoparticles, and engineered nanoparticles [11]. The large surface-to-volume ratio of nanoparticles, their ability of easy interaction with other particles, and several other features make them as an attractive tool in various fields. NPs are widely used in electronic, cosmetic, biomedical, purification of water and biotechnological applications. The synthesis of NPs can be achieved by some physical and chemical methods. In chemical synthesis, nanoparticles are grown in a liquid

medium containing various reactants particularly reducing agents such as sodium borohydride [12] and potassium bitartartrate [13]. Most commonly used chemical methods are chemical reduction [14], electrochemical techniques [15], and photochemical reactions in reverse micelles [16]. Commonly used physical methods are attrition and pyrolysis. Attrition involves grinding of the particles by a size-reducing mechanism. The particles are then air-classified, and oxidized nanoparticles are recovered. Pyrolysis involves burning of the precursor by passing them through an orifice at high pressure. The ash obtained is air classified to recover the oxidized nanoparticles [17]. The chemical methods available are often expensive, utilize lethal chemicals, and are comparatively complex. The demerits of physical methods are low production rate, high cost of production, and high energy consumption [18]. While biosynthesis of nanoparticles using biological agents such as bacteria, algae, fungi and plant extract has gained much attention in the area of nanotechnology in last few decades. The important advantages of biological systems, the low cost of cultivation, short production time, safety and the ability to up production volumes make plants an attractive platform for nanoparticle synthesis [19].

Algae have become significant organisms for biological purification of wastewater since they are able to accumulate plant nutrients, heavy metals, pesticides, organic and inorganic toxic substances and radioactive matters in their cells/bodies [20-24]. Algae are also able to accumulate highly toxic substances such as selenium, zinc and arsenic in their cells and/or bodies thus eliminating such substances from aquatic environments. Radiation is also an important type of pollution as some water contains naturally radioactive materials, and others become radioactive through contamination. Many algae can take up and accumulate many radioactive minerals in their cells even from greater concentrations in the water [25]. The ability of algae to accumulate metals and reduce metal ions makes them the superior contender for the biosynthesis of nanoparticles.

## 2. Types of Nanoparticles

There are two different types of NPs, inorganic NPs and organic NPs. The inorganic NPs include metal and metal oxides, which are potent antibacterial agents. Metal oxide nanoparticles such as silver (Ag), iron oxide ( $\text{Fe}_3\text{O}_4$ ), titanium oxide ( $\text{TiO}_2$ ), copper oxide ( $\text{CuO}$ ), and zinc oxide ( $\text{ZnO}$ ) are certain examples of inorganic NPs [26]. Organic NPs includes quaternary ammonium compounds, cationic quaternary polyelectrolytes, and chitosan. Organic nanoparticles are generally less stable at high temperatures. Due this reason, inorganic nanoparticles are more preferred [27]. Some inorganic NPs will be listed in details through this review.

### 2.1. Inorganic Nanoparticles

#### 2.1.1. Zinc Nanoparticles (ZnNPs)

ZnNPs have been given significant attention in water purification specifically arsenic removal [28], Cd (II) removal [29], Brown CGG dye [30], Organic dyes [31], Methylene

blue [32], Formaldehyde [33], Phenol [34], and Malachite green [35]. Owing to the wide applications of ZnNPs it has been synthesized by methods such as wet chemical method [36, 37], organic solvent method [38] and microwave method [39]. However biological synthesis using bacteria, fungi, algae and plant extracts is the method of choice and many researchers have been successful in synthesizing ZnNPs, for example using extract from microalgae *Cystophora moniliformis* [40].

#### 2.1.2. Silver Nanoparticles (AgNPs)

AgNPs have applications in non-linear optics, as intercalation materials for electrical batteries, optical receptors, as a catalyst and as an antibacterial. AgNPs can be applied to removal Congo red [41], Textile Effluent [42] and 4-nitrophenol [43]. The antimicrobial activity of AgNPs has many uses like the production of AgNPs coated blood collecting vessels, coated capsules etc. [44]. *Navicula atomus*, *Diademsis gallica*, *Stauroneis sp.*, *Sargassum wightii*, *Fucus vesiculosus* [45], *Caulerpa racemosa* [46] and *Sargassum muticum* [47] used in biosynthesis of AgNPs.

#### 2.1.3. Gold Nanoparticles (AuNPs)

AuNPs have many identified applications; can be produced in a number of geometries by chemical synthesis. AuNPs are biocompatible and hence can be used in disease diagnosis and therapy. AuNPs also can be used in remediation of methylene blue [48], methyl orange [49] and dichloromethane [50]. AuNPs have been biosynthesized from plant extracts, bacteria, fungi and algae such as *Sargassum muticum* [51] and *laminaria japonica* [52].

#### 2.1.4. Iron Nanoparticles (FeNPs)

FeNPs are considered to be the first nanoparticle used in environmental clean-up [53]. FeNPs can be easily synthesized, modified or coated and has super paramagnetic characteristics [54]. This property makes it easy to separate these super magnetic particles from aqueous solution and complicated matrices by applying an external magnetic field. Hence for application in various fields these nanoparticles have to be coated with either inorganic substances like silica, carbon or with organic species like surfactants and polymers [55]. FeNPs have been synthesized by various chemical and physical methods. Some emerging methods of synthesis of FeNPs are the use of algae such as *Sargassum muticum* [56] and plant extracts such as *Musa paradisiaca* [57] which exclude the use of harmful chemicals. FeNPs can be used in remediation process of various environmental pollutants such as Dissolved sulfides [58], Uranium [59], Nitrate [60], Cr (VI) [61], Ni (II) [62] and Cu (II), Pb (II) [63].

#### 2.1.5. Copper Nanoparticles (CuNPs)

Copper is one of the most widely used materials in the world due to its usage in fields like electricity, biomedical and antimicrobial applications. The nanoparticles synthesized from algae extracts were found to be covered by the medicinal properties of algae, for example biosynthesis of copper oxide nanoparticles using brown alga extract *Bifurcaria bifurcate* [64].

### 3. Biosynthesis of Nanoparticles Using Microalgae

#### 3.1. Synthesis of Silver Nanoparticles Using Chlorophyta, *Chlorella Vulgaris* [65]

AgNPs were biosynthesized using aqueous extract of *Chlorella vulgaris* as reducing agent and size of AgNPs synthesized ranged between 15 and 47 nm and analyzed for its antibacterial property against human pathogens.

The fresh water green algal strain of *Chlorella vulgaris* was collected from Algal Culture Collection, Center for Advanced Studies in Botany, University of Madras, India, and was inoculated in Bold Basal medium. The culture was maintained at 24°C in a thermostatically controlled room and illuminated with cool fluorescence lamps at an intensity of 2000 lux in a 16:8 h light/dark regime. In the exponential log phase, when the pigment, protein and carbohydrate measured were maximum, the cells were harvested. The collected cells were washed and sonicated using ultrasonic vibration at 30% amplitude for 20 min to release the water-soluble biomolecules. The homogenate was subjected to centrifugation (3–4 times), the supernatant was diluted through a series of dilutions with 1 mM AgNO<sub>3</sub>, and the reaction mixtures (10 mL) were incubated at 37°C. for AgNPs Characterization, UV–visible spectroscopy analysis, Scanning electron microscopy, Transmission electron microscopy, X-ray diffraction (XRD) measurement, and Fourier transform infrared (FTIR) were done. The antimicrobial activity of synthesized AgNPs was performed by using agar well diffusion method. About 20 mL of sterile molten Mueller–Hinton agar (HiMedia Laboratories Pvt. Limited, Mumbai, India) was poured into the sterile petriplates. Triplicate plates were swabbed with the overnight culture (10<sup>8</sup> cells/mL) of human pathogens: *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The solid medium was gently punctured with cork borer to make a well. Finally, the aqueous AgNPs (20 µL) were added into each well and incubated for 24 h at (37°C). After incubation, the zone of inhibition was measured and expressed as millimeter (mm) in diameter.

The absorption spectra of nanoparticles showed highly symmetric single band absorption with peak maximum at 421 nm with steadily increase in intensity as a function of time of reaction without any shift in intensity. After 72 h, no further increase in intensity was recorded, indicating the complete reduction in silver ions. Bio-reduced Ag<sup>+</sup> ions were further characterized for its size, shape, morphology and surface chemistry by SEM and TEM analyses. The large polycrystalline nature of the particles may be due to the fact that on nanometer scale most of the metals are as face-centered cubic (fcc) structures. They tend to nucleate and grow onto twinned and multiply twinned particles with their surfaces bounded by the lowest-energy (111) facets. AgNPs have the tendency to agglomerate due to their high surface tension of ultrafine nanoparticles. The fine particle size results in a large surface area that, in turn, enhances the nanoparticle

catalytic activity. Most of the particles had a size of about 27 nm. The AgNPs synthesized from aqueous algal extract are crystalline spherical particles. AgNO<sub>3</sub> complete reduction by aqueous *C. vulgaris* extract to crystalline AgNPs. The studies suggest that carbonyl groups of amino acids and peptides have stronger ability to bind metals, and the proteins could form a coat covering metal nanoparticles. The present finding coincides with the report suggesting that protein can bind to

Nanoparticles either through free amine groups, cysteine residues or through electrostatic attraction of negatively charged carboxylate groups in the cell-free extracts thus, the release of proteins probably has a role in the formation and stabilization of AgNPs in aqueous extract. The Antimicrobial activity of the silver, silver ions, silver compounds has been thoroughly investigated, and surveys have revealed the remarkable antibacterial activity of AgNPs. In our investigation, AgNPs were found to be toxic to human pathogens (*E. coli*, *P. vulgaris*, *S. aureus*, *P. aeruginosa* and *C. albicans*) and exhibited the maximum inhibition against *P. aeruginosa* of zone 21 mm followed by 20 mm against *Escherichia coli* AgNPs at 100 ppm totally inhibited the bacterial growth, but the activity against mold and dermatophytes was low; bacteria and molds exhibited resistance against AgNPs at 50 ppm concentration In case of *C. albicans* also, AgNPs are found to be cytotoxic disrupting the cell membrane and formation of pits and pores on membrane surface, subsequently leads to cell death.

#### 3.2. Synthesis of silver Nanoparticles Using Diatom, *Chaetoceros Calcitrans* [66]

*Chaetoceros calcitrans* was examined for its potential for synthesis of AgNPs. The characteristics of synthesized NPs were also studied by UV-Vis Spectrophotometer, FTIR and SEM. *Chaetoceros calcitrans* was isolated from Vellar estuary (11.4900° N, 79.7600° E), Parangipettai, India and their stock cultures was maintained in Conway and F/2 media respectively. The mass culture was performed with 40 L of culture media. The biomass was collected by centrifugation at 6000 rpm for 10 min under 4°C and lyophilized. For the preparation of algal extract, 10 mg of algal powders was suspended in 100 ml of double distilled water and filtered using Whatman No.1 filter paper. The filtrate was used for the synthesis of AgNPs. The reduction reaction of silver nitrate was started by mixing of 10 ml 10 mM AgNO<sub>3</sub> solution with 100 ml of algal extract. The reaction mixtures were heated at 60°C in hot air oven for one hour. Absorption of reaction mixtures were carried out using UNICAM UV 300 spectrophotometer at a resolution of 1 nm between 300 and 800 nm. For FTIR analysis, the reaction mixtures were centrifuged at 8000 rpm for 5 min., pellets were washed with double distilled water and centrifuged again. These pellets of algal colloid with AgNPs were freeze-dried and powdered using a micro pestle. FTIR spectrums of the samples were recorded on a Shimadzu IR Affinity-1 model in the range of 500–4000 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup>. To examine mean particle size and morphology of NPs, TESCAN, SEM machine was used. Briefly, the freeze dried sample of AgNPs

solution was sonicated with distilled water, thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were dried under a mercury lamp for 5 min and examined in SEM.

The color of the reaction mixtures were changed from yellowish green to dark brown after an hour of incubation at 60°C during the green synthesis using the extract of *C. calcitrans* due to surface plasmon resonance. The absorption of reaction mixture was observed between 350 and 600 nm by UV-Vis spectrometer a strong surface plasmon resonance was centered at 436 and 420 nm by *C. calcitrans* respectively. Surface Plasmon peak has also been well renowned for various metal NPs with sizes ranging widely from 2 to 100 nm. The size distribution and shape of synthesized AgNPs, the SEM analysis was executed and spherical shaped AgNPs were observed as deposition on algal extract. The size of AgNPs was ranged 30-35 nm from the extracts of *C. calcitrans*. The synthesized nanoparticles were predominantly spherical in shape; poly dispersed and had an average mean size of 22 nm. The SEM image of AgNPs in the present study, showed highly aggregated particles and the increased aggregation tendency of AgNPs in algae. FTIR analysis in the current study shows the involvement of functional groups like Amines, Phenols and Alcohols, Ethers and Aromatic rings in the reduction of silver ion to AgNPs and stabilization of AgNPs.

### 3.3. Synthesis of Silver Nanoparticles Using Cyanophyta, *Spirulina Platensis* [67]

Crystallized AgNPs have been biosynthesized by *Spirulina platensis* in an aqueous system. An aqueous solution of silver ions was treated with a live biomass of *Spirulina platensis* for the formation of AgNPs. *Spirulina platensis* strain PCC 9108 was obtained from the Culture Collection in France and was cultured in BG-11 medium. The AgNPs were prepared by taking 5 g of a thoroughly washed *Spirulina platensis* biomass from an exponential growth phase in a 250 ml Erlenmeyer flask with 100 ml of 1 mM aqueous  $\text{AgNO}_3$  solution (pH 7) for 24 h. The entire process of the reduction of metal ions to nanoparticles was carried out at 25°C. UV-Vis spectra analysis, TEM analysis of AgNPs and Powder X-Ray Diffraction (XRD) were done for Characterization of AgNPs.

The Extracellular synthesis of AgNPs has been shown from filamentous *Spirulina platensis*. It is well known that AgNPs exhibit a yellowish-brown color in aqueous solution biotransformation of ionic silver to reduced silver, and the subsequent formation of AgNPs in an aqueous medium observed that the maximum absorbance occurs at ca. 430 nm. The frequency peak of AgNPs by TEM comes at approximately 10–15 nm, and particles, whose sizes range from 5 to 30 nm, account for about 80% of the total particles observed TEM analysis showed that most particles had a size of ~12 nm. XRD analysis of the nanoparticles showed intense peaks, corresponding to (111), (200) and (220) Bragg reflection, based on the face-centered cubic (fcc) structure of AgNPs, with a lattice constant of  $a = 4.086$  whereas any peaks

originating from potential silver oxides ( $\text{AgO}$  or  $\text{Ag}_2\text{O}$ ) and  $\text{AgCl}$ , are absent. The size of crystallite in different planes of silver was determined as 17.8, 9.0 and 8.1 nm with the mean value of all three peaks as 11.6nm. Most probably, the reduction of AgNPs occurs due to the presence of cellular reductases released by *Spirulina platensis* into the solution. Also, in cyanobacteria, localized reducing conditions may be produced by a bacterial electron transport chain, via energy generating reactions within the cells. It seems that cyanobacteria have a high potential for synthesizing other nanoparticles with different shapes. For example, cubic AuNPs and octahedral gold plates produced from the filamentous cyanobacterium, *Plectonema boryanum* UTEX 485, are exposed to aqueous  $\text{Au}(\text{S}_2\text{O}_3)_2$  and  $\text{AuCl}_4$ , respectively.

### 3.4. Synthesis of Silver Nanoparticles Using Cyanophyta, *Oscillatoria Willei* [68]

The formation of AgNPs was investigated using silver nitrate in the presence of the marine cyanobacterium, *Oscillatoria willei* NTDM01. Marine cyanobacteria are one of the largest, photoautotrophic bacteria in marine ecosystem and are known to have high affinity transport system for nitrate. The marine cyanobacterial samples was collected and isolated from Kurusadai Island (Lat. 9° 18'N & Long 79° 10'E) at Gulf of Mannar, Tamilnadu, India. The culture was transferred and grown for approximately 4-6 weeks to reach a stationary growth phase. It was then centrifuged and washed several times with distilled water, deionized water to remove the trace metals from the surface of the cyanobacteria. To initiate the experiments, 5mL of silver solution (~560 mg/L) was added to 5mL of washed cyanobacterial cultures. The experiment were incubated at 25°C for 28 days and maintained in the dark. Abiotic experiments consisting of ~ 560mg/L silver were conducted using silver nitrate solution without the presence of cyanobacteria. The pH and cell viability were monitored during the course of the study. The sample have been characterized for the development of AgNPs by using Ultra Violet –Visible Spectrum (Uv-Vis), Fourier Transform Infra-Red (FTIR), Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopic (EDS) investigation.

On cyanobacteria experiment, the soluble silver was completely precipitated from solutions within 28 days. The UV-vis Spectrum reveals that the band observed at 450 nm due to the plasmon resonance of the AgNPs and low wavelength region recorded from the reaction medium exhibited an absorption band at ca.265 nm and it was attributed to aromatic amino acids of proteins. It is well known that the absorption band at ca. 265 nm arises due to electronic excitation in tryptophan and tyrosine residue in the protein. The FTIR spectrum was recorded from the film of AgNPs formed after several days of incubation with the bacteria. The bands seen at  $3280\text{ cm}^{-1}$  and  $2924\text{ cm}^{-1}$  were assigned to the stretching vibrations of primary and secondary amines respectively. The corresponding bending vibrations were seen at  $1651\text{ cm}^{-1}$  and  $1548\text{ cm}^{-1}$ , respectively. The two bands observed at  $1379\text{ cm}^{-1}$  and  $1033\text{ cm}^{-1}$  can be assigned to the

C–N stretching vibrations of aromatic and aliphatic amines, respectively. The presence of protein as the stabilizing agent surrounded the AgNPs. The silver ions were reduced in the presence of nitrate reductase, leading to the formation of a stable silver hydrosol 10–25 nm in diameter and stabilized by the capping peptide. At room temperature, the addition of  $\text{AgNO}_3$  to the cyanobacteria caused the precipitation of AgNPs at cell surfaces. Small spherical AgNPs with size ranging from 100 nm to 200 nm (extracellularly) were also precipitated in solution AgNPs were deposited at cell surfaces. The reaction of cyanobacteria with  $\text{AgNO}_3$  solutions results in the formation of AgNPs. The presence of spherical AgNPs was observed in experiment. The precipitation of AgNPs was not observed for abiotic experiment that were run under similar condition and duration, that suggesting that cyanobacteria were required for silver precipitation and the precipitation processes were not controlled by inorganic chemical reactions.

### 3.5. Synthesis of Silver Nanoparticles Using Cyanophyta, *Plectonema Boryanum* [69]

The extracellular and intracellular biosynthesis of spherical and octahedral AgNPs by the filamentous cyanobacterium, *Plectonema boryanum* UTEX 485, by incubating the algal extract from 25 to 100°C for up to 28 days have been reported. The size of the intracellular AgNPs was less than 10 nm and that of extracellularly synthesized AgNPs varied from 1 to 200 nm. The authors suggested that a possible mechanism for the intracellular bioreduction of  $\text{AgNO}_3$  at 25°C could be the cyanobacterial metabolic processes, utilizing nitrate ( $\text{NO}_3^-$ ) by reducing nitrate ( $\text{NO}_3^-$ ) to nitrite ( $\text{NO}_2^-$ ) and ammonium ( $\text{NH}_4^+$ ) which is incorporated in glutamine. Extracellular bioreduction was attributed to organic compounds (protein) released from dead cyanobacteria from 25 to 100°C.

### 3.6. Biosynthesis of AgNPs by Different Microalgae [70–76]

The synthesis of AgNPs with a change in coloration of the solution to brownish black using a diverse set of marine algae such as the chlorophytes *Tetraselmis gracilis* and *Chlorella salina*, the diatom *Chaetoceros calcitrans* and the haptophyte *Isochrysis galbana*. Two other microalgae, *Nannochloropsis oculata* and *C. vulgaris*, were also found to be capable of synthesizing AgNPs having a size smaller than 15 nm [73]. *Chlorella pyrenoidosa* also has been reported to synthesize spherical AgNPs in the size range of 5 to 10 nm; the AgNPs were extracted in 14 days and exhibited an absorption peak ( $\lambda_{\text{max}}$ ) at 450 nm [74]. In vitro and in vivo biosynthesis of rounded and rectangular AgNPs from *Chlamydomonas reinhardtii* [75]. The biosynthesis of AgNPs using fresh extracts and whole cells of the chlorophyte *Chlorococcum humicola* [76].

### 3.7. Synthesis of Gold Nanoparticles Using *Chlorella Vulgaris* [75]

The use of the green algae phytochemicals serves an easy and environmentally benign method of preparing AuNPs.

AuNPs synthesis by mixing an aqueous solution of chloroauric acid with cell-free aqueous extract of *C. vulgaris* complies with the green chemistry principles of using safe aqueous phytochemicals-based synthesis and extends its possible usage in the isotropy of spherical nanoparticles. The obtained AuNPs were comprehensively characterized for their average core size, morphology, purity, surface capping, crystal structure, and optical and bactericidal properties. *C. vulgaris* were collected from Algal Culture Collection, Center for Advanced Studies in Botany, University of Madras, Chennai, India and was inoculated in a Bold's Basal medium. When the pigment, protein and carbohydrate were measured to be maximum the cells were harvested. The collected cells were washed and sonicated using ultrasonic vibration at 30% amplitude for 20 min. The aqueous biomolecules were subjected to centrifugation (three to four times, at 5°C, 14,000 rpm, for 30 min).

The obtained *C. vulgaris* cell-free extract was diluted through a series of dilutions with 1 mM  $\text{HAuCl}_4$ . The reaction mixtures (10 mL) were put aside at 37°C and the reduction of the gold ions started at 1 mM  $\text{HAuCl}_4$ . The bioreduction and optical properties of the freshly prepared AuNPs were investigated by measuring the UV–Vis spectrum between 400 and 700 nm in a 10 mm path length quartz cuvette with a 1 nm resolution (UV–Vis, Beckman DU 64). TEM samples were prepared to measurement of the AuNPs and operated at an accelerating voltage of 30 kV using a CCD camera (Hitachi H-7600 AMT V600). The freshly prepared AuNPs with a glass substrate were subjected to XRD to measure the pattern (Model D/Max-2500) and scanning was executed in a 2 $\theta$  region from 30 to 80°. The pattern was recorded using Cu–K $\alpha$  radiation with a wavelength ( $\lambda$ ) of 1.5406 Å at a tube voltage of 40 kV and a tube current of 30 mA. FTIR analysis was carried out after the removal of the free biomolecules that were not adsorbed by the nanoparticles after repeated centrifugation and re-dispersion in water. Antimicrobial activity of the synthesized AuNPs was carried out by agar well diffusion method in triplicate against five human pathogens: *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

Aqueous extract of *C. vulgaris* is a reservoir of phytochemicals including pigments, astaxanthins, organic acids, amino acids, phenol, flavonoids, peptide and protein. In addition, the presence of carbohydrates (polysaccharides, oligosaccharides and reducing sugars) in the extract provides synergistic reducing power for the rapid transformation of chloroaurate ions into AuNPs. Different fractions of *C. vulgaris* cell-free extract reacted with  $\text{HAuCl}_4$  (1 mM) at 37°C. The analysis of the UV–visible spectrophotometric data confirmed that the surface plasma resonance (SPR) band was located at 530 nm for the AuNPs synthesized. The conversion of gold ions into AuNPs was found to be 90–95% at 37°C. The colors of all the test reaction mixtures changed from yellow to red, then black and the reaction completion was found to be dependent on the concentrations of the *C. vulgaris* fractions. The dark red color of the reaction mixture in all the studied test fractions indicates the formation of the

AuNPs.

Synthesized AuNPs were subjected to detailed characterization; to determine their sizes, morphologies, physical properties and surface chemistry and its toxicity to bacterial cells. The SEM and TEM analysis revealed that the AuNPs had an identical nature with an average size of about 10–2 nm. The crystalline nature of the AuNPs was evaluated via XRD. The angular positions of the diffracted Bragg peaks were observed. It was confirmed that the nanoparticles have a face-centered cubic structure with a lattice constant of 4.05 Å. The Bragg peaks equivalent to (111), (200), (220) and (311) demonstrate the formation of crystalline AuNPs. FTIR studies were carried out to identify the surface properties of the AuNPs. A major peak at 1,635 cm<sup>-1</sup> corresponds to amide I and amide II bonding from the capped peptides.

AuNPs were found to be toxic to human pathogens (*E. coli*, *P. vulgaris*, *S. aureus*, *P. aeruginosa* and *C. albicans*) and exhibited the maximum inhibition against *C. albicans* of zone 16 mm and *S. aureus* with 14 mm. Other three human pathogens were moderately susceptible. The capability of the *C. vulgaris* phytochemicals to efficiently reduce the chloroaurate ions into biocompatible AuNPs and its toxicity to human pathogens has thus been demonstrated. This single-step green method reveals *C. vulgaris* extract as a competent resource for both the manufacturing and nontoxic biomimetic capping of AuNPs and application in the medicinal field.

## 4. Biosynthesis of Nanoparticles Using Macroalgae

### 4.1. Synthesis of Silver Nanoparticles Using *Sargassum Cinereum* [76]

AgNPs synthesised by the aqueous extract of sea weed *Sargassum cinereum* was used as a reducing agent and its merits towards bactericidal activity against the pathogen *Staphylococcus aureus*, *Enterobacter aerogenes*, *Salmonella typhi* and *Proteus vulgaris*. Seaweed samples were freshly collected from the coastal areas of Goa, mainly Anjuna, Vagator and Dona Paula during May-June 2011 washed with distilled water and dried in an incubator for 2 days at 37°. Dried seaweed (25 g) was taken in a 500 ml conical flask added with 200 ml distilled water, boiled for 30 min and then filtered through Whatman no. 1 filter paper. This extract was dried with a rotary evaporator; 10 mg of the above was dissolved in 1 ml of sterile distilled water and stored in a refrigerator. Aqueous solution (1 mM) of silver nitrate (Sigma, MO, USA, 58157) was prepared in milli-Q water and used for synthesizing AgNPs. Seaweed extract (5 ml, 10%) was added to 45 ml of 1 mM silver nitrate for reduction in to Ag<sup>+</sup> ions. The mixed solution was subjected to vigorous stirring for 3 h using a magnetic stirrer. The reaction mixtures were continuously monitored for its colour change from yellowish green to orange. This indicates the formation of silver particles and the products were tested.

UV/Vis spectral analysis was done using Shimadzu

UV-2450 spectrophotometer with a resolution of 1 nm between 200 and 600 nm possessing the scanning speed of 500 nm/min. The reduction of Ag<sup>+</sup> ion was monitored by measuring the UV/Vis spectrum of the reaction medium. Nanoparticle solution showed the peak areas between 380 and 450 nm indicated the presence of AgNPs. To optimize the time required for the completion of nanoparticle formation, the reactions were monitored from 0 to 150 min. At every 30 min interval the solutions were removed from the reaction flasks and scanned between 200 and 600 nm with a spectrophotometer. Solutions containing the AgNPs were centrifuged and filtered using a 0.2 µm Whatman filter paper. The supernatant was taken on a glass plate and heat fixed using an incubator. The dried powder fixed to the glass plate was then mounted on the specimen holder and used for characterization studies. The sample was gold coated to make the sample conducting, using a sputtered and analyzed by a scanning electron microscope (SEM, Joel JSM- 6360A, Japan). SEM data were worked out with Image J software available in the internet to analyze the sizes of the nanoparticles.

Clinical isolates of both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Enterobacter aerogenes*, *Salmonella typhi* and *Proteus vulgaris*) bacterial strains were obtained from the Department of Microbiology, Goa Medical College and Hospital, Goa. The pathogens were maintained on nutrient agar (NA) (Hi-Media Laboratories Pvt. Ltd., Mumbai) and used for the antibacterial assay by standard disc diffusion method. Care was taken while handling these microbes in connection to biosafety and our institute instructions were followed strictly. Overnight grown inoculums 100 µl were spread plated on NA. Sterile paper discs of 5 mm diameter containing the AgNPs (i.e., 2.5, 5, 7.5 and 10 µl) equivalent to the concentrations of 25, 50, 75 and 100 µg/disc were placed in each plate. The plates were incubated at room temperature for 24 h and the zone of clearance around the disc was measured. Standard antibiotics like ampicillin (25 µg/disc); streptomycin (300 µg/ disc) and tetracycline (10 µg/disc) (Hi-Media, India) were also tested against the AgNPs to compare with.

*S. cinereum* extract showed color change from brown to reddish-yellow and had a peak at 342-408 nm, this may be due to the excitation of surface plasmon vibrations and it provides a convenient spectroscopic signature to indicate the formation of AgNPs. The nanoparticles synthesized using seaweed *S. cinereum* gave a particle size varying from 46 to 76 nm. The UV spectrum showed characteristic absorption at 408 nm for AgNPs, 220 nm for silver nitrate and 301 nm for sea weed extracts. The series of peaks show the formation of AgNPs of different sizes and shapes. The time for completion of the reaction was 3 h. As the duration of reaction increases, more AgNPs are formed. Due to the instability of the AgNPs formed an agglomeration may happened that leads to larger particle sizes. So the maximum time required for the completion of this process was stopped with 3 h. The formation of AgNPs has been observed only after 60 min. Before that there is no indication of nanoparticle formation.

When the time increased from 60 to 180 min with an interval of 30 min the peak area was increasing this indicate high amount of nanoparticle formations.

Antimicrobial activity tested with AgNPs synthesized from *S. cinereum* showed good activity against the pathogenic bacteria *Enterobacter aerogens*, *Proteus vulgaris* and *Salmonella typhi*. *Staphylococcus aureus* was shown slightly lower resistance while comparing with other pathogens. In general *S. cinereum* extract exhibited the zone of inhibitions from 10 to 29 mm. Even the minimum concentration of 2.5 µl sample tested could give the best activity against these pathogens. AgNPs synthesized by us were showing much better antibacterial activity against the multidrug-resistant organism *Enterobacter aerogens*. Equivalent resistances with standard ampicillin (25µg/disc) were shown by nanoparticles; the strain *Proteus vulgaris* observed to have more resistance to our nanoparticles than the ampicillin and slightly better than streptomycin (300µg/disc) and tetracycline (10µg/disc) *Staphylococcus aureus* indicated low resistance while comparing with the standard antibiotics tested. *Salmonella typhi* showed its resistance with our AgNPs in equivalent to standard antibiotics, in some cases it behaved much better resistance than the standards. The biosynthesized AgNPs using *S. cinereum* extract proved excellent antimicrobial activity and the activity is well demonstrated by considerable zone of inhibition against the multidrug resistant organisms *Enterobacter aerogens*, *Staphylococcus aureus*, *Salmonella typhi* and *Proteus vulgaris*.

#### 4.2. Synthesis Silver Nanoparticles Using *Padina Pavonica* [77]

The synthesis of pure and stable metallic nanoparticles of silver by the reduction of aqueous  $\text{Ag}^+$  ions with the thallus broth of marine alga, *P. pavonica* (Pahaeophyceae) and we further investigated the impact of the synthesized nanoparticles against *F. oxysporum* and *X. campestris* using agar well diffusion method. *Padina pavonica* was collected by hand picking method from the submerged marine rocks from Tuticorin district, Tamil Nadu during low tide at 6 AM. Collected algae were washed with tap water then shade-dried for two weeks, and powdered using domestic blender. 10 gram of the dried alga powder was boiled with 100 mL of deionized distilled water. The resulted infusion was filtered thoroughly until no insoluble material appeared in the alga leaf broth. The materials used for the synthesis of AgNPs are  $\text{AgNO}_3$  and algal thallus extract. Exactly 17 mg of  $\text{AgNO}_3$  was dissolved in 100 mL distilled water ( $10^{-3}\text{M}$ ). Ten mL of algal thallus extract was added to 90 mL of  $10^{-3}\text{M}$   $\text{AgNO}_3$  solution for reduction of  $\text{Ag}^+$  ions. The reduction of pure  $\text{Ag}^+$  ions was monitored by measuring the UV-vis spectra of the solution at regular intervals after diluting a small aliquot (0.2mL) of the sample 20 times. UV-vis spectra were recorded as a function of time of reaction on a UV- 1601 Shimadzu spectrophotometer with samples in Quartz cuvette operated at a resolution of 1 nm. X-ray diffraction (XRD) pattern of the alga thallus broth reduced AgNPs were obtained using Siemens D5005 XRD (X-ray diffractometer) with  $\text{CuK } \alpha$  radiation ( $\lambda = 0.1542$ ). XRD

patterns were analyzed to determine peak intensity, position and width. The particle size was calculated using the Scherrer formula:

$$d = 0.9\lambda / \beta \cos \theta$$

Where, d is the mean diameter of the nanoparticles,  $\lambda$ , the wavelength of X-ray radiation source and  $\beta$ , the angular FWHM of the XRD peak at the diffraction angle  $\theta$ . The alga thallus broth reduced AgNPs solution was centrifuged at 13,000 rpm for 15 minutes, re-dispersed in sterile distilled water to get rid of any uncoordinated biological molecules for Fourier transform infrared (FTIR) spectroscopy measurements. Centrifugation and the re-dispersion were repeated thrice in order to ensure better separation. The purified KBr pellets were then air dried at room temperature and powdered subjected to FTIR spectroscopy measurement (Shimadzu FTIR - 8300S). The morphology of the alga thallus broth reduced AgNPs was recorded using the JSM-6390 Scanning electron microscope (SEM). Samples for SEM were prepared by drop coating the AgNPs solutions onto carbon copper grid. The films on the grids were allowed to dry prior to SEM measurement. To record the size and shape of alga thallus broth reduced Ag nanoparticle, samples for Transmission Electron Microscopy (TEM) were prepared by drop-coating the Ag nanoparticle solution onto carbon-coated copper grids. The films on the TEM grids were allowed to stand for two minutes, following which the extra solution was removed using a blotting paper and the grid allow drying prior to measurement. TEM measurements were performed on a JEOL model 3010 instrument operated at an accelerating voltage at 120 kv.

Agar well diffusion bioassays were used to evaluate the microbicidal activity through the isolation of *Fusarium oxysporum* f. sp. *vasinfectum* and *Xanthomonas campestris* pv *malvacearum* from infected cotton plants and were used for the experiment. These pathogens were isolated, sub-cultured on Sabouraud Dextrose Agar (SDA) for fungi and Nutrient Agar (NA) for bacteria and identified using standard protocol [26]. Antimicrobial activity was carried out using agar well diffusion method. Petri plates were prepared with 20 mL each of sterile Mueller Hinton Agar (MHA) and SDA for bacteria and fungi respectively. Wells were made using sterile cork borer under aseptic condition. The alga thallus broth-based nanoparticles with various concentrations (25 µL, 50 µL, 75 µL, 100 µL) were added to the wells. Carbendazim (Bavistin) (0.03%) (BASF, Mumbai, India) and Chloramphenicol (0.1%) (HiMedia, Mumbai, India) were used as positive control for fungus and bacteria respectively and the distilled water was maintained as negative control for both microorganisms. The zone of inhibition was measured using a ruler and expressed in mm.

Terpenoids, phenolic compounds and saponins were observed in the alga thallus broth. The reaction of *P. pavonica* extract with  $\text{AgNO}_3$  solutions results in the formation of AgNPs observed in changing in color. The UV-vis spectra recorded from the *P. pavonica* reaction vessel at different times of reaction are showed strong surface plasmon



resonance centered at 422 nm clearly indicated an increase in intensity with time and stability after 24 h of reaction. A number of Bragg reflections with  $2\theta$  values of  $38.03^\circ$ ,  $46.18^\circ$ ,  $63.43^\circ$  and  $77.18^\circ$  sets of lattice planes are observed which may be indexed to the 111, 200, 220 and 311 facets of silver respectively. X-Ray diffraction pattern thus clearly illustrates that the AgNPs formed in this present synthesis are crystalline in nature and the size was found to be  $\sim 54$  nm. The metallic silver nano-crystals showed typically optical absorption peak approximately at 3 KeV. The (FTIR) observed peaks were more characteristic of terpenoids that are very abundant in algal thallus broth. The presence of terpenoids in algal thallus broth was also confirmed by phytochemical analysis. The peaks observed in crude algal thallus broth at  $1415.65\text{ cm}^{-1}$ . The alga thallus broth synthesized nanoparticles were spherical with sizes ranged from 45 to 64 nm. TEM images recorded from drop-coated films of the AgNPs synthesized by treating silver nitrate solution with alga thallus broth for 24 h. the AgNPs formed were predominantly spherical and poly dispersed with diameters in the range 10 to 72 nm (mean value = 46.8 nm). The AgNPs inhibited the growth of *F. oxysporum* and *X. campestris*. However, aqueous crude extract of *P. pavonica* showed no activity against these pathogens, hence it can be concluded that the antimicrobial activity is due to the presence of AgNPs.

#### 4.3. Synthesis of Silver Nanoparticles Using *Caulerpa Racemosa* [78]

The seaweed *Caulerpa racemosa* extract was used for the synthesis of  $\text{AgNO}_3$  with sizes in the range of 10 nm and also assessed their antagonistic effect against gram-positive and gram-negative bacteria.

Sample collection and Preparation of seaweeds extract Green seaweed *C. racemosa* was collected from the Gulf of Mannar, Southeast coast of India. The samples were thoroughly washed, dried and ground well. 1 g of biomass was kept in a 250-ml conical flask with 100 ml of Milli Q water for 24 h. Finally, the extract was filtered and stored it in a refrigerated temperature for further analysis. for the biosynthesis of AgNPs 10 ml seaweed filtrate was added in 90 ml of  $10^{-3}$  M aqueous  $\text{AgNO}_3$  solutions at room temperature the bio-reduction of silver nitrate into AgNPs can be confirmed by visual observation. The bacterial strains *Staphylococcus aureus* (ATCC 29123) and *Proteus mirabilis* (ATCC 25933) were obtained from American Type of Culture Collection Centre (ATCC). UV-visible spectroscopy analysis, X-ray diffraction (XRD) measurement, The Fourier transforms infrared (FT-IR) measurements and Transmission electron microscopy (TEM), were done for the Characterization of AgNPs. The antibacterial activity was assayed by using the agar well diffusion test technique. Muller Hinton agar medium (MHA) was prepared, 20 ml of the sterilized media was poured into sterilized petri dishes and allowed to solidify at room temperature. A sterile cotton swab is used for spreading each test microorganism from the 24 h inoculated broth evenly on the MHA plates and left for a few minutes to allow complete absorption of the inoculums. In

each of these plates 5-mm diameter wells were made at the center using an appropriate size sterilized cork borer. Different concentrations of algal extract were added to the respective wells on the MH agar plates. Concentration ranges from 5, 10 and 15  $\mu\text{l}$ , respectively, were placed in the wells and allowed to diffuse at room temperature for 30 min. No AgNPs was added in the control plate. The AgNPs loaded plates were kept in incubation at  $37^\circ\text{C}$  for 24 h. After incubation, a clear inhibition zone around the wells indicated the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses.

The absorption spectra of the as-prepared Nano sized silver samples were characterized by UV-visible spectroscopy. *C. racemosa* has proved to be an important biological component for the extracellular biosynthesis of stable AgNPs. It is well known that AgNPs exhibit light yellowish to brown color. UV-Vis spectra of the silver nitrate solutions incubated with marine green algae as a function of time of reaction. The surface plasmon resonance (SPR) band of Nano silver occurs initially at 440 nm (3 h). It is observed that the Nano silver SPR band is centered at about 413 nm. From the spectra, it is clear that when the function of reaction time increased, the SPR band is shifted towards shorter wavelength region which shows a decrease in particle size as a result of increased band gap. At lower concentrations, the SPR band is broad and it is due to large anisotropic particles. A smooth and narrow absorption band at 413 nm is observed which is characteristic of almost spherical nanoparticles. The position of SPR band in UV-Vis spectra is sensitive to particle shape, size, its interaction with the medium, local refractive index and the extent of charge transfer between medium and the particles. FT-IR spectra were recorded for *C. racemosa* extract and synthesized AgNPs to identify the possible biomolecules responsible for the reduction of  $\text{AgNO}_3$  into AgNPs. FT-IR spectrum of *C. racemosa* shows different major peaks positioned at 3416, 2924, 2854, 1631, 1389, 1061, 1019 and  $660\text{ cm}^{-1}$ . On the other hand, FT-IR spectrum of the synthesized AgNPs shows the presence of major peaks at 3440 and  $1639\text{ cm}^{-1}$ . Thus, the peptides may play an important role in the reduction of  $\text{AgNO}_3$  into AgNPs. The XRD pattern of synthesized AgNPs was observed and compared with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS). Intense diffraction peaks due to AgNPs are clearly observed at  $38.24^\circ$ , and  $44.42^\circ$ ,  $64.44^\circ$  and  $77.40^\circ$  are pertaining to the (111) (200), (220) and (311) planes of Bragg's reflection based on the FCC (JCPDS, file No. 04-0783) structure of AgNPs. No reflection peaks related to nitrate ions and other impurities were observed in this pattern. In addition, the acquired reflections are sharp with good intensity which confirms that the structures of synthesized nanoparticles are well crystalline. The morphology of AgNPs is almost spherical with few triangular nanoparticles. From the histogram analysis, it is noted that the particles with the size of 10 nm was more pronounced. The results of antibacterial activity with a zone of inhibition maximum was found in *P. mirabilis* (14 mm for 15  $\mu\text{l}$ ) and minimum level antibacterial activity present in *S. aureus* (7



mm for 5  $\mu$ l). The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. When AgNPs enter the bacterial cell, it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity.

#### 4.4. Synthesized Silver Nanoparticles Using *Ulva Lactuca* [79]

Synthesized AgNPs by reduction of aqueous  $\text{Ag}^+$  ions with the thallus of marine alga *Ulva lactuca*. Synthesized particles were characterized by various techniques like UV-Vis spectroscopy, SEM, TEM, XRD and FTIR. Also, the antibacterial ability of the AgNPs was tested against bacterial isolates. Seaweed samples (25gm) were ground with 100ml of sterile distilled water for 5 min. The crude extract was passed through Whatman No.1 filter paper and the filtrate was stored at 4°C for further use. 1mM aqueous solution of Silver nitrate ( $\text{AgNO}_3$ ) was prepared and used for the synthesis of AgNPs. 10 ml of *Ulva lactuca* extract was added into 90 ml of aqueous solution of 1 mM Silver nitrate for reduction into  $\text{Ag}^+$  ions and kept at room temperature for 72 hrs. at 120 rpm. Suitable controls were maintained throughout the experiments. UV-Vis spectroscopy measurements, Transmission electron microscopic analysis, XRD measurements, and The FTIR spectra of algae extract synthesized AgNPs were analyzed for the characterization of AgNPs. The AgNPs synthesized using *U. lactuca* was tested for antimicrobial activity by agar well-diffusion method against bacterial strains *Bacillus* sp., *E.coli* and *Pseudomonas* sp. The pure cultures of bacterial strains were subcultured on nutrient agar medium. Wells of 10 mm diameter were made on nutrient agar using gel puncture. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, different concentrations of the sample of nanoparticles solution (10, 20, 30, 40, and 50  $\mu$ l) was poured onto each well on all three plates. After incubation at 37°C for 24 hours, the different levels of zone of inhibition of bacterial strains were measured.

It is well known that AgNPs exhibit reddish brown color in aqueous solution due to excitation of surface plasmon vibrations in AgNPs. As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from pale yellow to reddish brown due to reduction of silver ion which indicated formation of AgNPs. Absorption spectra of AgNPs formed in the reaction media had absorbance peak at 423nm, The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium. The AgNPs synthesized by *Ulva lactuca* extract were scanned using TEM from which the average mean size of the AgNPs was 20 nm and seems to be spherical in morphology. The X-ray diffraction peaks were found to be broad around their bases indicating that the silver particles are in nanosizes. The peak

broadening at half maximum intensity of the X-ray diffraction lines. The XRD spectrum of AgNPs exhibiting the characteristic peaks of the silver crystallites observed at  $2\theta$  values of 38.5°, 44.48°, 64.69° and 77.62° corresponding to (111), (200), (220) and (311) of the face centered cubic AgNPs. FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. Absorbance bands seen at 3448, 1627 and 1384  $\text{cm}^{-1}$  were assigned to the NH stretching vibrations of free NH group, C=C stretching vibrations of non-conjugated group and CH bending vibrations of  $\text{CH}_3$  group, The results revealed that the capping ligand of the AgNPs may be an aromatic compound or alkanes or amines. The inhibition zone was observed against *Bacillus* sp., *Pseudomonas* sp. and *E.coli*. In the quantitative assay, 10, 20, 30, 40, and 50  $\mu$ l of AgNPs were added in five respective wells. The zone of inhibition was measured as 6, 7, 8, 9 and 10 mm respectively in diameter.

#### 4.5. Synthesis of AgNPs Using Different Macroalgae [80-86]

Spherical and polydispersal crystalline AgNPs with an average size of 40.5 nm were biosynthesized with, *Ulva fasciata*. Also, the antibacterial activity of the synthesized AgNPs against *Xanthomonas campestris* was observed with a MIC of  $40 \pm 5.77 \mu\text{g mL}^{-1}$ . Fresh and dry extracts of *Codium capitatum* have also been used for biosynthesis of AgNPs of size range 3 to 44 nm and with an average size of 30 nm [80]. The aqueous extract of the green alga, *Caulerpa racemosa* in  $10^{-3}$  M  $\text{AgNO}_3$  solution at RT was found to biosynthesize spherical along with some triangular AgNPs of 5 to 25 nm size. The AgNPs exhibited antibacterial activity against human pathogens, *Proteus mirabilis* and *S. aureus*. The biosynthesis of AgNPs using aqueous extracts of the brown alga, *Sargassum plagiophyllum*, and the green algae, *Ulva reticulata* and *Ulva compressa*.

It was observed that heating aqueous extract of algae with  $\text{AgNO}_3$  solution at 60°C for 15 min produced good results, forming spherical AgNPs with a size range of 20 to 50 nm [81]. The extracellular biosynthesis of AgNPs by brown alga, *Padina pavonica* [82]. When 10 mL of the algal extract solution was incubated along with 90 mL of 1 mM  $\text{AgNO}_3$ , spherical crystalline AgNPs ranging from 45 to 64 nm were formed in 24 h.

The AgNPs showed antibacterial activity against the cotton pathogens *Fusarium oxysporum* and *X. campestris*. Spherical AgNPs with an average size of 14 nm employing brown alga, *Padina tetrastratica*, after incubation of  $\text{AgNO}_3$  solution and algal extract for 24 h [83]. These AgNPs exhibited highest antibacterial activity against *Pseudomonas* sp. followed by *B. subtilis* and *Klebsiella planticola*. Moreover, sodium alginate extracted from *P. tetrastratica* was used to reduce  $\text{AgNO}_3$  solution and the AgNPs thus formed were found to be stable for 72 h possessing significant antibacterial activity against multidrug-resistant bacterial strains like *S. aureus* and *P. aeruginosa*. Synthesis of stable AgNPs with a  $\lambda_{\text{max}}$  at 410 nm when aqueous extract of *Padina gymnospora* was incubated with  $\text{AgNO}_3$  solution with continuous shaking at 30°C [84].

The AgNPs were spherical and their size ranged from 25 to 40 nm which significantly inhibited growth of gram-positive *Bacillus cereus* and gram-negative *E. coli* bacteria. AgNPs were biosynthesized using *Sargassum muticum* aqueous extract. Amount of 50 mL of 1% aqueous extract was added to 50 mL of 1 mM AgNO<sub>3</sub> for 30 min at 35°C followed by keeping the reaction mixture stable at RT for 2 h. Spherical AgNPs with a size range of 5–15 nm and with a  $\lambda_{\text{max}}$  of 420 nm were formed within 20 min. Sulphated polysaccharides within the algae were attributed for the reduction of Ag<sup>+</sup> [85].

Spherical AgNPs were obtained of an average size of 22 nm using the aqueous extract of the red alga *Gelidiella aceros* [85]. The spherical-shaped AgNPs present in the filtrate were well distributed as non-aggregates and showed a broad  $\lambda_{\text{max}}$  peak at 408 nm. Aromatic compounds or alkanes or amines were attributed to be the capping ligand of the AgNPs. These AgNPs were a potent antifungal agent against pathogenic fungal strains analogous to a commercial antifungal agent, *clotrimazol*, with high antifungal activity against *Mucor indicus* and *Trichoderma reesei* and moderate activity against *Fusarium dimerum* and *Fusicola insolens*. Agar extracted from the red alga *Gracilaria dura* has also been reported to reduce AgNO<sub>3</sub> solution, forming spherical AgNPs with an average size of 6 nm [86].

#### 4.6. Synthesis of Gold Nanoparticles Using, *Sargassum wightii* [87]

This is the first report in synthesis of highly stable AuNPs by the reduction of aqueous AuCl<sub>4</sub><sup>-</sup> by the extract of marine alga *Sargassum wightii*. *S. wightii* were collected from Mandapam Camp south east coast of Tamil Nadu, India. They were cleaned and then shade dried for 3–5 days then ground by a glass mortar. Material used for the synthesis of AuNPs are chloroauric acid (HAuCl<sub>4</sub>) (Loba Chemicals) was used as received. Formation of Au<sup>0</sup> was carried out by taking 1 g of seaweed powder in a 500mL Erlenmeyer flask with 100mL of 10<sup>-3</sup>M aqueous HAuCl<sub>4</sub> solution. The 95% of the bioreduction of AuCl<sub>4</sub><sup>-</sup> ions occurred within 12 h at stirring condition. Aliquots of the reaction solution were removed and absorptions were measured using a UV-1601 Shimadzu spectrophotometer operated at a resolution of 1 nm. Samples analysis by high-resolution transmission electron microscopic (HR-TEM) and X-ray diffraction (XRD).

The color of the reaction mixtures change of the medium to ruby red after 15 h of incubation. The light absorption pattern of the algal biomass was kinetically monitored in the range of 300–800 nm. UV–vis spectra were recorded from the aqueous chloroauric acid and algae reaction medium. In the case of gold ions reduction, the bands corresponding to the surface plasmon resonance (SPR) occurred at 527 nm. The TEM micrographs of the AuNPs formed predominantly mono disperse with diameter ranging from 8 to 12 nm. On careful observation of is various magnifications of TEM images of AuNPs it is noted that the particles are of uniformed size ca. around 11 nm, and also AuNPs have an inclination of forming thin planner structures than spherical structures. The XRD patterns thus clearly show that the AuNPs formed by the

bio-reduction of AuCl<sub>4</sub><sup>-</sup> ions using *S. wightii*.

## 5. Conclusions

Through this review we attempted to summarize the previous studies for algae-mediated biosynthesis of metallic nanoparticles providing further knowledge of this applied science. The synthesis of highly stable gold and silver NPs has been successfully synthesized by different micro and macro algae. The unique characteristics of nanoparticles have made them the particle of choice in many fields including remediation of environmental pollutants, degrading pollutants like pesticides, dyes, hydrocarbons, TCE etc. Antimicrobial nanoparticles offer various distinctive advantages in reducing acute toxicity, overcoming resistance, and lowering cost, when compared to conventional antibiotics. Ecofriendly biosynthesis of nanoparticles using green resources is a simple, environmentally friendly, pollutant-free and low-cost approach. Furthermore, it remains to be evaluated which particular alga is preferable to reduce a particular metal type with a higher yield.

## References

- [1] Edhaya Naveena, B.; Prakash, S. 2013: Biological synthesis of gold nanoparticles using marine algae *Gracilaria corticata* and its application as a potent antimicrobial and antioxidant agent. *Asian J. Pharm. Clin. Res.* 6(2):179–182.
- [2] Madhuri, S.; Maheshwar, S.; Sunil, P.; Oza, G. 2012: *Nanotechnology concepts and applications*, vol. 4. CRC Press, USA.
- [3] Sutherland, K. 2008: What is nanofiltration? *Filtration and Separation* 45(8), 32-35. [This presents approaches to the study of nanofiltration]
- [4] Eriksson P. 1988: Nanofiltration extends the range of membrane filtration. *Environmental Progress*, 7(1), 58–62. [This presents approaches to the study of nanofiltration technology]
- [5] Ventresque, C.; Gisclon, V.; Bablon, G.; Chagneau, G. 2000: An outstanding feat of modern technology: the Mery-sur-Oise Nanofiltration Plant. *Desalination*, (131) 1-16. [This presents approaches to the case study of a nanofiltration plant]
- [6] Hassaan, M. A., and Ali, H. R. 2017: *Fresh Water Pollution and Heavy Metals Removal*. 1st Edition, Publisher: Lambert Academic publishing, e-book [ISBN: 978-3-659-57770-3]. pp. 220.
- [7] Ali, H. R and Hassaan, M. A., 2017: Applications of Bio-waste Materials as Green Synthesis of Nanoparticles and Water Purification, *Advances in Materials*. Vol. 6, No. 5, pp. 85-101. doi: 10.11648/j.am.20170605.16
- [8] Ali, H. R. and Hassaan, M. A. 2017: *Different Techniques for Water Purification*. 1st Edition, Publisher: Lambert Academic publishing, e-book [ISBN: 978-3-659-55636-4]. pp. 217
- [9] Hassaan, M. A., El Nemr, A., and Madkour, F. F. 2017a: Testing the advanced oxidation processes on the degradation of Direct Blue 86 dye in wastewater. *Egypt. J. Aquat. Res.* 43, 11–19.

- [10] Hassaan, M. A., El Nemr, A., and Madkour, F. F. 2017b: Advanced oxidation processes of Mordant Violet 40 dye in freshwater and seawater. *Egypt. J. Aquat. Res.* 43, 1–9.
- [11] Buzea, C.; Pacheco II; Robbie, K. 2007: Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases* 2 (4): 17–71.
- [12] Kim, J. S.; Kuk, E.; Yu KN; Kim, J.; Park, S. J.; Lee, H. J. 2007: Antimicrobial effects of silver nanoparticles. *Nanomed Nanotechnol Biomed* 3: 95–101.
- [13] Tan, Y.; Dai, X.; Li, Y.; Zhu, D. 2003: Preparation of gold, platinum, palladium and silver nanoparticles by the reduction of their salts with a weak reductant-potassium bitartrate. *J. Mater. Chem.* 13: 1069–1075.
- [14] Balantrapu, K.; Goia, D. V. 2009: Silver nanoparticles for printable electronics and biological applications. *J. Mater Res* 24 (9): 2828–2836.
- [15] Rodriguez-Sánchez, L.; Blanco, M. C.; Lopez-Quintela, M. A. 2000: Electrochemical synthesis of silver nanoparticles. *J. Phys. Chem. B.* 104: 9683–9688.
- [16] Taleb, A.; Petit, C.; Pileni, M. P. 1997: Synthesis of highly monodisperse silver nanoparticles from AOT reverse micelles: a way to 2D and 3D self-organization. *Chem Mater* 9 (4): 950–959.
- [17] Chen, S.; Zhao, X.; Xie, H.; Liu, J.; Duan, L.; Ba, X.; Zhao, J. 2012: Photoluminescence of undoped and Ce-doped SnO<sub>2</sub> thin films deposited by sol–gel-dip-coating method. *Appl. Surf Sci.* 258: 3255–3259.
- [18] Li, Y.; Duan, X.; Qian, Y.; Li, Y.; Liao, H. 1999: Nanocrystalline silver particles: synthesis, agglomeration, and sputtering induced by electron beam. *J Colloid Interface Sci.* 209 (2): 347–349.
- [19] Njagi, E. C.; Huang, H.; Stafford, L.; Genuino, H.; Galindo, H. M.; Collins, J. B.; Hoag, G. E.; Suib, S. L.; Langmuir: 2011. V. 27. № 1. P. 264–271.
- [20] Kalesh, N. S.; Nair, S. M. 2005: The Accumulation Levels of Heavy Metals (Ni, Cr, Sr, & Ag) in Marine Algae from Southwest Coast of India. *Toxicological & Environmental Chemistry* 87(2): 135-146.
- [21] Jothinayagi, N.; Anbazhagan, C. 2009: Heavy Metal Monitoring of Rameswaram Coast by Some *Sargassum* species. *American-Eurasian Journal of Scientific Research*; 4 (2): 73-80.
- [22] Alp, M. T.; Sen, B.; Ozbay, O. 2011: Heavy Metal Levels in *Cladophora glomerata* which Seasonally Occur in the Lake Hazar. *Ekoloji*, 20 (78): 13-17.
- [23] Alp, M. T.; Ozbay O, Sungur, M. A. 2012: Determination of Heavy Metal Levels in Sediment and Macroalgae (*Ulva* sp. and *Enteromorpha* sp.) on the Mersin Coast 2011. *Ekoloji* 21, 82, 47-55.
- [24] Labib, W.; Abou Shady, A. M.; Laila, A. M.; El Shafaay and Shimaa Hosny 2015: Ecological studies of macroalgae in Alexandria Mediterranean waters, Egypt. *J. Exp. Biol. (Bot.)*, 11(2): 169–180.
- [25] Palmer, C. M. 1969: A composite rating of algae tolerating organic pollution. *J. Phycology*; 5: 78-82.
- [26] Loomba, L.; Scarabelli, T.; 2013: Metallic nanoparticles and their medicinal potential. Part II: aluminosilicates, nanobiomagnets, quantum dots and cochleates. *Ther Deliv* 4(9): 1179–1196.
- [27] Jain, A.; Duvvuri, L. S.; Farah, S.; Beyth, N.; Domb, A. J.; Khan, W. 2014: Antimicrobial polymers. *Adv Healthcare Mater* 3: 1969–1985.
- [28] Singh, N.; Singh, S. P.; Gupta, V.; Yadav, H. K.; Ahuja, T.; Tripathy, S. S. 2013b: A process for the selective removal of arsenic from contaminated water using acetate functionalized zinc oxide nanomaterials. *Environ Prog Sustain Energy* 32(4):1023–1029.
- [29] Srivastava, V.; Gusain, D.; Sharma, Y. C. 2013: Synthesis, characterization and application of zinc oxide nanoparticles (n-ZnO). *Ceram Int* 39(8):9803–9808.
- [30] Islam, A. A.; Ferdous, T.; Das, A. K. 2015: Photodegradation of brown CGG dye using ZnO nanoparticles synthesized by ionic template method.
- [31] Sanna, V.; Pala, N.; Alzari, V.; Nuvoli, D.; Carcelli, M. 2016: ZnO nanoparticles with high degradation efficiency of organic dyes under sunlight irradiation. *Mater Lett* 162:257–260.
- [32] Jain, N.; Bhargava, A.; Panwar, J. 2014: Enhanced photocatalytic degradation of methylene blue using biologically synthesized “protein-capped” ZnO nanoparticles. *Chem. Eng. J.* 243:549–555.
- [33] Darvishi Cheshmeh, Soltani, R.; Rezaee, A.; Safari, M.; Khataee, A. R.; Karimi, B. 2015: Photocatalytic degradation of formaldehyde in aqueous solution using ZnO nanoparticles immobilized on glass plates. *Desalin Water Treat* 53(6):1613–1620.
- [34] Kruefu, V.; Ninsonti, H.; Wetchakun, N.; Inceesungvorn, B.; Pookmanee, P.; Phanichphant, S. 2012: Photocatalytic degradation of phenol using Nb-loaded ZnO nanoparticles. *Eng J-CANDA* 16 (3):91–100
- [35] Khezami, L.; Taha, K. K.; Ghiloufi, I.; El Mir, L. 2016: Adsorption and photocatalytic degradation of malachite green by vanadium doped zinc oxide nanoparticles. *Water Sci Technol* 73(4): 881–889.
- [36] Lee, J.; Easteal, A. J.; Pal, U.; Bhattacharyya, D. 2009: Evolution of ZnO nanostructures in sol–gel synthesis. *Curr Appl Phys* 9(4):792–796.
- [37] Mehta, S. K.; Singh, K.; Umar, A.; Chaudhary, G. R.; Singh, S. 2012: Ultra-high sensitive hydrazine chemical sensor based on low-temperature grown ZnO nanoparticles. *Electrochim Acta* 69:128–133.
- [38] Mezni, A.; Kouki, F.; Romdhane, S.; Warot-Fonrose, B.; Joulié, S.; Mlayah, A.; Smiri, L. S. 2012: Facile synthesis of ZnO nanocrystals in polyol. *Mater Lett* 86:153–156.
- [39] Nehru, L. C.; Swaminathan, V.; Sanjeeviraja, C. 2012: Rapid synthesis of nanocrystalline ZnO by a microwave-assisted combustion method. *Powder Technol* 226:29–33.
- [40] Vigneshwaran, N.; Kumar, S.; Kathe, A. A.; Varadarajan, P. V.; Prasad, V. 2006: Functional finishing of cotton fabrics using zinc oxide-soluble starch nanocomposites. *Nanotechnology* 17(20):5087.
- [41] Modi, S.; Pathak, B.; Fulekar, M. 2015: Microbial synthesized silver nanoparticles for decolorization and biodegradation of azo dye compound. *J. Environ Nanotechnol* 4(2):37–46.

- [42] Corso, C. R.; De Almeida ACM 2009: Bioremediation of dyes in textile effluents by *Aspergillus oryzae*. Microb Ecol 57(2):384–390.
- [43] Gangula, A.; Podila, R.; Karanam, L.; Janardhana, C.; Rao, A. M. 2011: Catalytic reduction of 4-nitrophenol using biogenic gold and silver nanoparticles derived from *Breynia rhamnoides*. Langmuir 27(24):15268–15274.
- [44] Geoprincy, G.; Saravanan, P.; Gandhi, N. N., Renganathan S 2011: A novel approach for studying the combined antimicrobial effects of silver nanoparticles and antibiotics through agar over layer method and disk diffusion method. Dig J Nanomater Biostruct 6(4):1557–1565.
- [45] Asmathunisha, N.; Kathiresan, K. 2013: A review on biosynthesis of nanoparticles by marine organisms. Colloids Surf B 103:283–287.
- [46] Kathiraven, T.; Sundaramanickam, A.; Shanmugam, N.; Balasubramanian, T. 2015: Green synthesis of silver nanoparticles using marine algae *Caulerpa racemosa* and the antibacterial activity against some human pathogens. Appl Nanosci 5(4):499–504.
- [47] Azizi, S.; Namvar, F.; Mahdavi, M.; Ahmad, M. B.; Mohamad, R. 2013: Biosynthesis of silver nanoparticles using brown marine macroalga *Sargassum muticum* aqueous extract. Materials 6 (12):5942–5950.
- [48] Suvith, V. S.; Philip, D. 2014: Catalytic degradation of methylene blue using biosynthesized gold and silver nanoparticles. Spectrochim Acta, Part A 118:526–532.
- [49] Soomro, R. A.; Nafady, A. 2015: Catalytic reductive degradation of methyl orange using air resilient copper nanostructures. J. Nanomater 2015:120.
- [50] Huang, C. C.; Lo, S. L.; Lien, H. L. 2012: Zero-valent copper nanoparticles for effective dechlorination of dichloromethane using sodium borohydride as a reductant. Chem Eng J 203:95–100.
- [51] Namvar, F.; Azizi, S.; Ahmad, M. B.; Shameli, K.; Mohamad, R.; Mahdavi, M. Tahir, P. M. 2015: Green synthesis and characterization of gold nanoparticles using the marine macroalgae *Sargassum muticum*. Res Chem Intermed 41(8):5723–5730.
- [52] Ghodake, G.; Lee, D. S. 2011: Biological synthesis of gold nanoparticles using the aqueous extract of the brown algae *Laminaria japonica*. J Nanoelectron Optoe 6(3):268–271.
- [53] Tratnyek, P. G.; Johnson, R. L. 2006: Nanotechnologies for environmental cleanup. Nano Today 1 (2):44–48.
- [54] McHenry, M. E.; Laughlin, D. E. 2000: Nano-scale materials development for future magnetic applications. Acta Mater 48(1):223–238.
- [55] Wu, S. H.; Mou, C. Y.; Lin, H. P. 2013: Synthesis of mesoporous silica nanoparticles. Chem Soc Rev 42 (9):3862–3875.
- [56] Mahdavi, M.; Namvar, F.; Ahmad, M. B. Mohamad, R. 2013: Green biosynthesis and characterization of magnetic iron oxide (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles using seaweed (*Sargassum muticum*) aqueous extract. Molecules 18(5):5954–5964.
- [57] Herlekar, M.; Barve, S.; Kumar, R. 2014: Plant-mediated green synthesis of iron nanoparticles. J. Nanopart.
- [58] Chaung, S. H.; Wu, P. F.; Kao, Y. L.; Yan, W.; Lien, H. L 2014: Nanoscale zero-valent iron for sulfide removal from digested piggy wastewater. J Nanomater.
- [59] Fan, F. L.; Qin, Z.; Bai, J.; Rong, W. D.; Fan, F. Y.; Tian, W.; Zhao, L. 2012: Rapid removal of uranium from aqueous solutions using magnetic Fe<sub>3</sub>O<sub>4</sub>@ SiO<sub>2</sub> composite particles. J Environ Radioact 106:40–46.
- [60] Ryu, A.; Jeong, S. W.; Jang, A.; Choi, H. 2011: Reduction of highly concentrated nitrate using nanoscale zero-valent iron: effects of aggregation and catalyst on reactivity. Appl Catal B 105 (1):128–135.
- [61] Cutting, R. S.; Coker, V. S.; Telling, N. D.; Kimber, R. L.; Pearce, C. I.; Ellis, B. L.; Arenholz, E. 2010: Optimizing Cr (VI) and Tc (VII) remediation through nanoscale biomineral engineering Environ Sci Technol 44(7):2577–2584.
- [62] Li, X. Q.; Zhang, W. X. 2006: Iron nanoparticles: the core-shell structure and unique properties for Ni (II) sequestration. Langmuir 22(10): 4638–4642.
- [63] Mahdavian, A. R.; Mirrahimi, M. A. S. 2010: Efficient separation of heavy metal cations by anchoring polyacrylic acid on superparamagnetic magnetite nanoparticles through surface modification. Chem. Eng. J 159(1):264–271.
- [64] Abboud, Y.; Saffaj, T.; Chagraoui, A.; El Bouari, A.; Brouzi, K.; Tanane, O.; Ihssane, B. 2014: Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (*Bifurcaria bifurcata*). App Nanosci. 4 (5):571–576.
- [65] Jayshree Annamalai and Thangaraju Nallamuthu 2016: Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency, Appl. Nanosci., 6:259–265.
- [66] Karthikeyan, P.; Mohan, D.; Abishek, G. and Priya, R. 2015: Synthesis of silver nanoparticles using Phytoplankton and its characteristics, International Journal of Fisheries and Aquatic Studies. 2(6): 398–401.
- [67] Mahdieh, M., Zolanvari, A., Azimeea, A. S. Mahdieh, M. 2012: Green biosynthesis of silver nanoparticles by *Spirulina platensis*, Scientia Iranica, 19 (3), 926–929.
- [68] Mubarak Ali, D.; Sasikala, M.; Gunasekaran, M. and Thajuddin, N. 2011: Biosynthesis and characterization of Silver Nanoparticles using marine cyanobacterium, *Oscillatoria willei* NTDM01. Digest Journal of Nanomaterials and Biostructures. 6, (2): 385–390.
- [69] Aishwarye Sharma, Shruti Sharma, Kuldeep Sharma, Siva P. K., Chetri, Amit Vashishtha, Pushpa Singh, Ravindra Kumar, Brijesh Rathi and Veena Agrawal, 2016: Algae as crucial organisms in advancing nanotechnology a systematic review; J Appl. Phycol. 28:1759–1774.
- [70] Merin, D. D.; Prakash, S.; Bhimba, B. V. 2010: Antibacterial screening of silver nanoparticles synthesized by marine micro algae. Asian Pac J Trop Med 3:797–799.
- [71] Mohseniazar, M.; Barin, M.; Zarredar, H.; Alizadeh, S.; Shanehbandi, D. 2011: Potential of microalgae and Lactobacilli in biosynthesis of silver nanoparticles. BioImpacts 1:149–152.
- [72] Elumalai, S.; Santhos, B. I.; Devika, R.; Revathy, S. 2013: Collection, isolation, identification, and biosynthesis of silver nanoparticles using microalga *Chlorella pyrenoidosa*. Nanomechanics Sci Technol Int J 4:59–66.

- [73] Barwal, I.; Ranjan, P.; Kateriya, S.; Yadav, S. C. 2011: Cellular proteins of *Chlamydomonas reinhardtii* control the biosynthesis of silver nanoparticles oxido-reductive. *J Nanobiotechnol* 9:1–12.
- [74] Jena, J.; Pradhan, N.; Dash, B. P.; Sukla, L. B.; Panda, P. K. 2013: Biosynthesis and characterization of silver nanoparticles using microalga *Chlorococcum humicola* and its antibacterial activity. *Int J Nanomater Bios* 3:1–8.
- [75] Jayshree Annamalai and Thangaraju Nallamuthu, 2015: Characterization of biosynthesized gold nanoparticles from aqueous extract of *Chlorella vulgaris* and their anti-pathogenic properties, *Appl. Nanosci.*, 5:603–607.
- [76] Mohandass, C.; Vijayaraj, A. S.; Rajasabapathy, R.; Satheeshbabu, S.; Rao, S. V.; Shiva, C. and De-mello, L.; 2013: Biosynthesis of Silver Nanoparticles from Marine Seaweed *Sargassum cinereum* and their Antibacterial Activity, *Indian J Pharm Sci.*;75(5):606-610.
- [77] Sahayaraj, K.; Rajesh, S.; and Rathi, J. M. 2012: silver nanoparticles biosynthesis using marine alga *Padina pavonica* (LINN.) and its microbial activity, *Journal of Nanomaterials and Biostructures*, 7(4): 1557-1567.
- [78] Kathiraven, T.; Sundaramanickam, A.; Shanmugam, N. and Balasubramanian, T. 2014: Green synthesis of silver nanoparticles using marine algae *Caulerpa racemosa* and their antibacterial activity against some human pathogens, *Appl Nanosci.* DOI 10.1007/s13204-014-0341-2.
- [79] Sangeetha, N., Saravanan, K. 2014: Biogenic Silver Nanoparticles using Marine Seaweed (*Ulva lactuca*) and Evaluation of its Antibacterial activity, *Journal of nanoscience and nanotechnology*. 2 (1): 99-102.
- [80] Kannan, R. R. R.; Stirk, W. A.; Staden, J. V. 2013: Synthesis of silver nanoparticles using the seaweed *Codium capitatum* P. C. Silva (Chlorophyceae). *S Afr J Sci Bot* 86:1–4.
- [81] Dhanalakshmi, P. K.; Azeez, R.; Rekha, R.; Poonkodi, S.; Nallamuthu, T. 2012: Synthesis of silver nanoparticles using green and brown seaweeds. *Phykos* 42:39–45.
- [82] Sahayaraj, K.; Rajesh, S.; Rathi, J. M. 2012: Silver nanoparticles biosynthesis using marine alga *Padina pavonica* (Linn.) and its microbicidal activity. *Dig J Nanomater Biostruct* 7:1557–1567.
- [83] Sangeetha, N.; Manikandan, S.; Singh, M. Kumaraguru, A. K. 2012: Biosynthesis and characterization of silver nanoparticles using freshly extracted sodium alginate from the seaweed *Padina tetrastromatica* of Gulf of Mannar, India. *Curr Nanosci* 8: 697–702.
- [84] Shiny PJ, Mukherjee A, Chandrasekaran N (2013) Marine alga-mediated synthesis of the silver nanoparticles and its antibacterial efficiency. *Int J Pharm Sci* 5:239–241.
- [85] Marimuthu, V.; Palanisamy, S. K.; Sesurajan, S.; Sellappa, S. 2011: Biogenic silver nanoparticles by *Gelidiella acerosa* extract and their antifungal effects. *Avicenna J Med Biotechnol* 3:143–148.
- [86] Shukla, M. K.; Singh, R. P.; Reddy, C. R. K.; Jha, B. 2012: Synthesis and characterization of agar-based silver nanoparticles and nanocomposite film with antibacterial applications. *Bioresour Technol* 107:295–300.
- [87] Singaravelu, G.; Arockiamary, J. S.; Ganesh Kumar, V.; Govindaraju, K. 2007: A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. *Colloids and Surfaces B: Biointerfaces* 57 (97–101).